Normally, organisms exhibit wide variation in their degree of attachment to solid supports. Certain organisms, for example, readily cling or attach to a wide variety of supports, including both organic and inorganic materials, while others will only attach to supports of biological origin (attachment-dependent organisms). Other organisms exhibit little direct attachment to any support material (attachment-independent organisms). The microsponge of the present invention, because it is prepared from polymeric (organic) materials and because of its permeability (porosity and pore structure) should be suitable for immobilizing substantially all types of organisms.

In fact, as noted in more detail below, it even is possible to tailor the micro-structure or configuration of the microsponge to best accommodate the attachment tendency of the immobilized organism. For example, microsponges having the wire-mesh structure (Figure 1) can be employed in conjunction with attachment-independent organisms, while microsponges exhibiting a leafy structure (Figure 2) can be used with attachment dependent organisms.

Any suitable procedure used by the prior art for immobilizing such organisms on microsponges can be used in the present invention including such techniques as adsorption and chemical coupling. For example, in the case of certain organisms it will only be necessary to mix the collagen microsponges in a broth inoculated with the specific organism. After a short period of time, the organism will colonize the microsponges and become entrapped in their pores. In the case of some organisms such as fibroblasts and hybridomas, it also may be desirable to coat the microsponge with attachment-promoting materials such as fibronectin, polylysine and anti-hybridoma antibodies prior to inoculation. Other techniques, such as applying a net charge to the surface of the microsponge, also can be used to enhance immobilization.

As will be recognized by those skilled in this art, in the broad practice of the present invention, the procedure used for bringing the immobilized bioactive material into direct contact with a liquid reagent stream such as a growth supporting medium for culturing of immobilized organisms is not critical and any of the numerous arrangements available in the prior art including such well known apparatus as stirred tank reactors, fixed bed reactors, fluidized bed reactors and moving bed reactors and the like could be used. Generally, when culturing organisms the microsponges are charged to a suitable reactor and mixed therein with a nutrient broth and an inoculum of the organism. The microsponges should be completely submerged. The microsponges are incubated so that the organisms grow and colonize the porous matrix of the microsponge. Fresh nutrient broth along with other materials necessary for growth, such as oxygen in the case of aerobic organisms, are supplied in a continuous manner to the reactor and harvest liquor containing the biochemical product of interest is recovered. The biochemical product may comprise a primary or secondary metabolite of an immobilized organism, excess biomass generated by an immobilized organism contalning for example a non-secretory product, an immobilized enzyme catalyzed reaction product or the like.

A particular advantage of the microsponge of the present invention is that it can be used in a mixed or motive system such as a fluidized bed reactor. As used herein, the term "motive reactor" refers to reactor systems in which relative motion between the microsponge and the fluid medium is provided in part by imparting motion to the microsponges themselves. Such reactor systems substantially enhance mass and energy transfer. A particularly preferred motive reactor system is described in co-pending U.S. Patent Application Serial No. 706,872 filed on February 28, 1985 in the names of Robert C. Dean, Jr., Peter V. Grela and Subhash B. Karkare.

To prepare a highly crosslinked collagen microsponge, a suitable collagen source first is milled to a small "fiber" size. Generally, the collagen is milled (e.g., using a Wiley mill) to obtain particles (fibers) with a maximum dimension on the order of about 200 microns. Preferably, the collagen source material is milled (i.e., dry ground) to yield fibers having a diameter on the order of 1 to 50 microns and a length no greater than about 200 microns. Proper milling of the collagen source material is important for obtaining microsponges of the desired structure.

The milled collagen then is formed into a collagen-based solution or dispersion, i.e., a soluble collagen dissolved in a solvent, or an insoluble collagen dispersed in a solvent by admixture with a suitable solvent, particularly acids such as dilute hydrochloric acid, dilute acetic acid or the like. In the present invention organic acids are particularly preferred, including acetic acid, lactic acid, proprionic acid, butyric acid and the like. Certain long chain fatty acids also could be used. The collagen is mixed into the liquid (solvent) using standard mixing equipment. Preferably, in the case of a collagen dispersion the mixing is accomplished with a high level of agitation using, for example, a Waring blender, so as to produce microfibers of the collagen. The mixture of collagen and solvent typically comprises between about 0.5% to about 1.5% by weight of the collagen. The mixture preferably exhibits a pH in the range of about 2.0 to about 4.0. A pH in the range of 1.0 to 2.0 may also be used as long as the temperature of the mixture

is sufficiently reduced (e.g., about 4 °C) to avoid denaturization of the collagen.

Next, the weighting additive is blended with the collagen-liquid mixture and the composite mixture is formed into small droplets and rapidly solidified by freezing at a temperature below about 0 °C and preferably below about -30 °C to form particles of the desired size. Any known technique for producing small particles can be employed in carrying out the present invention. Suitable techniques include, inter alia, pressure or air-shear spraying, emulsification techniques, droplet formation using Raleigh liquid jet instability techniques, extrusion techniques, droplet formation using gravity or centrifugal forces, electrostatic droplet formation, and droplet formation using inertial forces. For example, suitably sized particles have been prepared using inertial forces to form small droplets at the orifice of a vibrating needle. The droplets can be frozen by allowing them to fall into a cryogenic bath of liquid nitrogen. Obviously other chilling baths for freezing the droplets could be used, e.g., chilled ethanol. Also, larger sized particles formed by freezing possibly could be reduced to the desired particle size by such destructive techniques as grinding and the like. Still additional techniques such as various coating methodologies, could be used to form microsponges having a solid core of the weighting additive. In this case a shell of the collagen matrix would surround the weighted core. Those skilled in the art will recognize other techniques suitable for forming small particles of the types described above and the present invention is not intended to be limited to any specific technique.

The pore size and structure of the collagen microsponge is influenced by a variety of factors. For example, changes in the collagen concentration appear to affect pore size, with higher collagen concentrations tending to yield smaller pore dimensions. The pH of the mixture and the specific acid used in preparing the mixture also affect the pore size and structure of the resultant microsponge. For example, it has been found that too low a pH tends to significantly limit the pore dimensions of the microsponge while higher pHs cause a distinct collagen phase to separate from the original solution or dispersion thereby preventing the formation of a porous structure and when a finely divided weighting additive is used it tends to remain in the dispersed phase. The rate of freezing also appears to influence the structure of the microsponge and the structure also will vary with changes in collagen type.

Thereafter, the frozen composite is vacuum freeze-dried preferably using conventional equipment operating at a vacuum of more than about 50 millitorr and at a temperature in the range of about 22 °C to -100 °C. The combination of freezing and drying is referred to as lyophilization.

The freeze-dried collagen matrix composite then is treated so as to crosslink the collagen. The collagen can be crosslinked using either chemical crosslinking agents preferably selected from the group consisting of a carbodiiamide or N-hydroxy succinimide-derived active esters (succinimidyl active esters), by severe dehydration at an elevated temperature or by a combination of these treatments. The strength and biostability of the collagen matrix so-prepared is influenced by the degree of crosslinking introduced through such treatment. These crosslinking methods provide a collagen matrix that is surprisingly resistant to collagenase and other enzymatic degradation thereby making these materials particularly suitable for culturing organisms. Examples of carbodiiamides which can be used in the chemical treatment are cynamide and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiiamide hydrochloride. Suitable bifunctional succinimidyl active esters include bifunctional N-hydroxy succinimide, 3,3'-dithio(sulfosuccinimidyl) proprionate and bis(sulfosuccinimidyl) suborate. When using such chemical crosslinking agents, the dry collagen matrix material is immersed in a solution of the crosslinking agent at about room temperature for a period of time of from about 2 to 96 hours. The solution of crosslinking agent may contain from about 0.1 to about 15% (weight per volume) of the crosslinking agent. Alternatively, the crosslinking agent could be added to the original solution or dispersion of the collagen source.

To crosslink the collagen matrix using severe dehydration, the microsponge is subjected to a vacuum of about 50 millitorr or more for a period of time of from about 2 to about 96 hours at a temperature in the range of about 50 °C to about 200 °C, e.g., about 100 ° to 110 °C.

As noted above, the two treatments also can be used in combination and it is preferred to use a severe dehydration treatment followed by chemical treatment to crosslink the collagen matrix. Generally, however, in those cases where chemical treatment precedes the dehydration treatment the crosslinking agent should be added directly to the original collagen solution or dispersion prior to formulation of the matrix particles and lyophilization in order to facilitate subsequent vacuum dehydration treatment. Also as noted above the strength and biostability of the collagen matrix is influenced by the degree of crosslinking introduced through such treatment. These crosslinking methods provide a collagen matrix that is surprisingly resistant to collagenase and other enzymatic degradation thereby making these materials particularly suitable for culturing organisms. Whenever chemical treatment is used, the collagen matrix should be washed exten-

sively prior to further use in order to remove any excess crosslinking agent. Further information concerning the procedure for preparing the highly crosslinked collagen can be obtained from U.S. Serial No. 593,733 filed on March 17, 1984 in the names of Frederick H. Silver, Richard A. Berg, David E. Birk, Kevin Weadock and Conrad Whyne and entitled "Biodegradable Matrix and Methods for Producing Same," the disclosure of which is incorporated herein by reference.

After thoroughly washing the crosslinked collagen matrix in ultra-pure water, the microsponges then may be sterilized using conventional sterilization techniques. A particular advantage of the collagen microsponges is that they are manufactured separate from the step of organism immobilization and as a result they can be properly sterilized prior to being inoculated or stored. Preferably, the microsponges are sterilized using gamma irradiation. Ethylene oxide also may be used as an alternative, as may additional sterilization procedures known to those skilled in the art, as long as the important characteristics of the microsponge are not compromised. Obviously, when sterilizing the microsponges using ethylene oxide the particles must be thoroughly ventilated in order to remove all traces of this sterilizing agent before subsequently using the microsponges for culturing organisms. It also has been discovered that the severe dehydration treatment for an extended time at an elevated temperature used to crosslink the collagen may satisfactorily sterilize the microsponge, thus obviating any additional treatment.

Preferably, the sterilized microsponges are aseptically packaged for delivery to the ultimate consumer. The user simply places the microsponges into a previously sterilized reactor, adds the proper nutrients and inoculum and initiates operation. In a preferred embodiment, the package actually comprises a disposable reactor vessel having the necessary connections for feeding a nutrient stream, for removing a harvest liquor and for ancillary operations, as needed, such as heat exchange, oxygenation and process control. For a fluidized bed reaction, the vessel also would contain a suitably designed distribution plate. Such a pre-packaged disposable reactor vessel may have a volume between about 0.1 liter and 10 liters. In this case, the user of the reactor simply integrates it into the process equipment consisting of pumps, valves, piping, heat and gas exchangers and various instrumentation and related probes and begins operation. Providing a disposable reactor, pre-packaged with the microsponges sterilized and ready for use, significantly simplifies start-up procedures for culturing organisms, particularly when changing from one culture to another.

Although not completely understood, it has been observed that variations in process parameters lead to important variations in the structure or configuration of the highly crosslinked collagen matrix itself. Figures 1 and 2, which are photomicrographs of the collagen matrix prepared using the techniques described above, illustrate these different structures. The photomicrographs of Figures 1 and 2 were obtained using scanning electron microscopy. Figure 1 illustrates a collagen matrix having substantially a wire-mesh structure. In this structure, the diameter of the fiber network typically is on the order of about 1 micron. This structure is particularly desirable for attachment-independent type organisms such as hybridomas. In this matrix, such organisms become trapped in and on the matrix structure. Figure 2 illustrates a leaf-type matrix structure. The leaves of this structure typically have a thickness on the order of about 1 micron. This structure is particularly suitable for attachment-dependent cells such as fibroblast cells. Currently, it is believed that the rate of chilling (freezing) the droplets in the bead making process influences the morphology of the collagen microsponge.

The following examples are intended to more fully illustrate the invention without acting as a limitation on its scope.

Example 1

This example describes the preparation of microsponges of the highly crosslinked collagen matrix material.

Partially purified tendon collagen was milled to obtain fibers having a length of less than about 200 microns and a diameter of between about 5 and 50 microns using a Wiley Mill obtained from VWR Scientific. An amount of milled collagen material then was mixed with a solution of acetic acid using a Waring blender so as to produce a collagen-based dispersion having a pH of about 3.0 and about 1.0% (by weight) of collagen. Then, an inert weighting material, titanium, was added to the collagen-based dispersion as a fine powder having particle sizes within the range of about 5 to about 180 microns.

The composite mixture then was formed into solid particles by first producing small droplets of the composite mixture. Droplets were produced by flowing the composite mixture through a vibrating hollow needle having an internal diameter of about 1.3 millimeters vibrated at a frequency of about 90 Hz. The droplets fell into a cryogenic bath of liquid nitrogen and were rapidly frozen. The frozen droplets of the composite mixture then were vacuum dried using a Virtis Freezemobile Lyophilizer Model 6 at a vacuum of about 10 millitorr for about

48 hours.

After such lyophilization, the dried microsponges were subjected to a severe dehydration (dehydrothermal treatment) at a temperature of about 100 °C under a vacuum of about 10 millitorr for about 72 hours using a VWR Scientific drying oven. The microsponges then were treated with a 1.0% (by weight) solution of cyanamide as a chemical crosslinking agent at a pH of about 5.5 for about 24 hours at about 20 °C.

The highly crosslinked microsponges then were thoroughly washed for about 24 hours using ultra-pure water, were dried and then sterilized by gamma irradiation. The microsponges had particle sizes within the range of about 200 to 800 microns, a void fraction on the order of about 77%, pore sizes on the order of about 20 microns, and a specific gravity on the order of about 1.1. The microsponges had a wire mesh micro-structure.

Example 2

The microsponges of Example 1 can be used to support the growth of hybridoma cells. In particular, about 300 ml of the microsponges can be contained in a 600 ml reactor vessel. The microsponges can be inoculated with the hybridoma cells and cultured using a suitable nutrient medium. The reactor can be operated at a solids concentration of about 25-40%, while the content of the reactor is vigorously agitated. A nutrient medium such as Delbecko Modified Eagle medium with 10% fetal calf serum can be passed into the reactor in a continuous manner and a product stream containing the monoclonal antibodies can be recovered at a substantially equivalent flow rate.

It will be obvious to one of ordinary skill that numerous modifications may be made without departing from the true spirit and scope of the invention which is to be limited only by the appended claims.

Claims

1. A microsponge for immobilizing bioactive materials in motive bioreactor systems, being characterized in that the microsponge comprises a porous, biostable matrix of a biocompatible polymer containing an inert weighting material which weights the microsponge; the matrix having an open to the surface pore structure with an average pore size in the range of from about 1 to about 150 microns, the pores of the matrix occupying from about 70 to about 98% by volume of the microsponge, and in that the microsponge has an average particle size of from about 100 to 1000 microns and a specific gravity of above about 1.05.

2. A microsponge according to claim 1 having immobilized therein bioactive material selected from the group consisting of enzymes, microorganisms, dead cells and living cells.

3. A microsponge according to claim 1 wherein said biostable, biocompatible polymer is selected from collagen, cellulose, dextran, dextrin, polyamides, polyesters, starch, agarose, carrageenin, polyurethanes, polyvinyl alcohols, polyacrylates, polymethacrylates and polyacrylamides.

4. A microsponge according to claim 1 wherein the inert weighting material is selected from metals, metal alloys, metal oxides and ceramics.

5. A microsponge according to claim 4 wherein the weighting materials has a specific gravity of above about 4.0 and the microsponge has a specific gravity of above about 1.3.

6. A microsponge according to claim 5 wherein the inert weighting material is dispersed throughout the porous matrix as finely divided powder.

7. A microsponge according to claim 5 wherein the weighting material is centrally disposed as a solid core about which the porous matrix is formed.

8. A microsponge according to claim 5 wherein the inert weighting material is selected from chromium, tungsten, cobalt, molybdenum, titanium, nickel and alloys.

9. A microsponge according to claim 8 wherein the weighting material is titanium and the microsponge has hybridoma cells immobilized therein.

10. A microsponge according to claim 1 wherein the bioactive biocompatible polymer is highly crosslinked collagen.

11. A microsponge according to claim 10 wherein the highly crosslinked collagen matrix is prepared by the steps of
    (a) milling a source of collagen selected from Type I, II and III collagen,
    (b) admixing the milled collagen with an acidic liquid medium,
    (c) lyophilizing the collagen-liquid medium mixture into a dry matrix, and

(d) crosslinking the collagen in the dry matrix by a treatment selected from
(i) contacting the collagen with a crosslinking agent selected from the group consisting of carbodiimides and bifunctional suiccinimidyl active esters,
(ii) subjecting the solid droplet to elevated temperatures under a vacuum, and
(iii) a combination thereof.

12. A microsponge according to claim 11 wherein the inert weighting material is added into the mixture of collagen and acidic liquid medium as a finely divided powder prior to lyophilization.

13. The microsponge of claim 12 wherein the collagen is Type 1, tendon collagen.

14. A bioreactor system comprising a plurality of weighted microsponges according to claim 1 sterilized and aseptically sealed in a reactor vessel.

15. A bioreactor system according to claim 14 wherein the reactor has a volume between about 0.1 to 10 litres.

16. A bioreactor system according to claim 15 wherein the reactor is a fluidized bed reactor, having a fluid distribution plate.

17. A process for performing a bioreaction comprising immobilizing a bioactive material in the microsponges of claim 1; containing the microsponges having the immobilized bioactive material in a suitable reactor vessel; passing a liquid reagent stream into the reactor in direct contact with the microsponges; agitating the mixture of the microsponges and the reagent stream and recovering the biochemical reaction products from the reactor.

18. A process according to claim 17 wherein organisms are immobilized in the microsponges, the microsponges are incubated to promote growth and colonization of the microsponges by the organisms, the reagent comprises nutrient media for promoting the growth and metabolism of the organisms, and wherein the product comprises metabolites of the organisms.

19. A process of claim 17 wherein organisms are immobilized on the microsponges and the recovered product comprises free organisms which have escaped from the microsponges.

20. A process according to claim 18 wherein the organisms comprise hybridomas and the product comprises monoclonal antibodies.

21. A process according to claim 20 wherein the reactor vessel comprises a fluidized bed reactor.

22. A process according to claim 18 wherein the organisms comprise mammalian cells and the products comprise mammalian cell products.

23. A process according to claim 18 wherein the organisms are genetically engineered microbial cells and the product comprises secreted protein products.

24. A process according to claim 19 wherein the organisms are genetically engineered microbial cells and the product comprises the organisms containing a non-secreted protein product.

25. A method for the continuous in vitro culture of organisms for the production of biochemical products comprising the steps of:
(a) providing a highly crosslinked, collagen microsponge prepared by
(i) milling a source of collagen selected from the group consisting of Types I, II and III collagen,
(ii) admixing the milled collagen with an acidic liquid medium,
(iii) lyophilizing the collagen-liquid medium mixture into a dry sponge matrix, and
(iv) crosslinking the collagen in the sponge matrix by a treatment selected from:
(1) contacting the collagen with a crosslinking agent selected from the group consisting of carbodiimides and bifunctional succinimidyl active esters,
(2) subjecting the dry sponge matrix to elevated temperatures under a vacuum, and
(3) a combination thereof;
(b) inoculating the highly crosslinked, collagen microsponge of step (a) with a culture of the organisms;
(c) passing a nutrient media in direct contact with the inoculated, highly crosslinked collagen microsponge; and
(d) recovering the biochemical products with the nutrient media effluent.

26. A method according to 25 wherein the organisms are selected from bacteria, fungi, viruses, algae, yeasts, animal cells and plant

cells.

27. A method of claim 25 wherein the organisms are mammalian cells and the biochemical products comprise mammalian cell products.

28. A method according to claim 25 wherein the organisms are hybridomas and the biochemical products comprise monoclonal antibodies.

29. A method according to claim 25 wherein the organisms are genetically engineered and the biochemical products comprise the organisms containing a non-secreted protein product.

30. A method according to claim 25 wherein the organisms are genetically engineered and the biochemical products comprise secreted protein products.

31. A method according to claim 25 wherein the highly crosslinked, collagen microsponge is formed into a shape selected from beads, flakes, discs, fibres, films and sheets.

32. A method according to claim 25 wherein said highly crosslinked, collagen microsponge is provided as a coating on a support.

33. A method according to claim 25 wherein the highly crosslinked, collagen microsponge has an open to the surface pore structure with an average pore size in the range of from about 1 to about 150 microns and the pores of the microsponge occupy from about 70 to about 98% by volume of the matrix.

34. A method according to claim 25 wherein the highly crosslinked, collagen microsponge is in the shape of a bead with an average particle size of from about 100 to 1000 microns.

35. A method of claim 25 wherein the nutrient media is passed in direct contact with the inoculated, highly crosslinked collagen microsponge in a reactor selected fixed bed reactors, stirred tank reactors, fluidized bed reactors and moving bed reactors.

36. A method according to claim 25 wherein the highly crosslinked collagen in the microsponge has a molecular weight of from about 1×10^6 to about 50×10^6.

37. A method according to claim 25 wherein the highly crosslinked collagen has a molecular weight between crosslinks of from about 1,000 to 100,000 via covalent bonding.

Patentansprüche

1. Ein Mikroschwamm zur Immobilisierung/Festlegung bioaktiven Materials in bewegten Bioreaktor-Systemen, dadurch gekennzeichnet, daß der Mikroschwamm besteht aus einer porösen, biostabilen (biologisch stabilen) Matrix aus biokompatiblem (biologisch verträglichem) Polymer, welches ein inertes Beschwerungsmaterial zur Beschwerung des Mikroschwamms enthält; die Matrix hat eine zur Oberfläche offene Porenstruktur mit einer durchschnittlichen Porengröße von ungefähr 1 bis ca. 150 Mikrons, die Poren der Matrix nehmen ungefähr 70 Vol-% bis 98 Vol-% des Volumens des Mikroschwammes ein (ca. 70 bis 98 Vol-% Porenvolumen), und daß der Mikroschwamm eine durchschnittliche Partikelgröße von ungefähr 100 bis 1000 Mikrons und ein spezifisches Gewicht von ungefähr 1,05 hat.

2. Ein Mikroschwamm nach Anspruch 1 mit darin immobilisiertem bioaktivem Material, welches ausgewählt ist aus der Gruppe bestehend aus Enzymen, Mikroorganismen, toten Zellen und lebenden Zellen.

3. Ein Mikroschwamm nach Anspruch 1, wobei das biostabile, biokompatible Polymer ausgewählt ist aus den Stoffgruppen Collagenen, Zellulose, Dextran, Dextrin, Polyamiden, Polyestern, Stärke, Agarose, Carragenin, Polyurethan, Polyvinyl-Alkohol, Polyacrylat, Polymethacrylat und Polyacrylamid.

4. Ein Mikroschwamm nach Anspruch 1, wobei das inerte Beschwerungsmaterial ausgewählt ist aus Metallen, Metallegierungen, Metalloxyden und Keramiken.

5. Ein Mikroschwamm nach Anspruch 4, wobei das Beschwerungsmaterial ein spezifisches Gewicht von ungefähr 4.0 und der Mikroschwamm ein spezifisches Gewicht von ungewähr 1,3 hat.

6. Ein Mikroschwamm nach Anspruch 5, wobei das inerte Beschwerungsmaterial dispergiert bzw. fein verteilt ist über die poröse Matrix als fein verteiltes Pulver.

7. Ein Mikroschwamm nach Anspruch 5, wobei das Beschwerungsmaterial zentral angeordnet ist als ein fester Kern, um den herum die poröse Matrix gebildet ist.

8. Ein Mikroschwamm nach Anspruch 5, wobei das inerte Beschwerungsmaterial/Gewichtsmaterial ausgewählt ist von Chrom, Wolfram, Kobald, Molybdän, Titan, Nickel und deren Legierungen.

9. Ein Mikroschwamm nach Anspruch 5, wobei das Gewichtsmaterial Titan ist und Hybridoma-Zellen in den Mikro-Schwamm immobilisiert sind.

10. Mikroschwamm nach Anspruch 1, wobei das bioaktive, biokompatible Polymer in hohem Maße über Kreuz verbundenes Collagen ist.

11. Mikroschwamm nach Anspruch 10, wobei die kreuzweise verbundene Collagen-Matrix gebildet ist durch die Verfahrensstufen:
(a) Mahlen eines Collagenmaterials ausgewählt von den Typen I, II und III Collagen,
(b) Vermischen des gemahlenenen Collagens mit einem sauren flüssigen Medium,
(c) Gefriertrocknen der flüssigen Collagenmischung in eine trockene Matrix und
(d) kreuzweises Verbinden des Collagens in der trockenen Matrix durch eine Behandlung ausgewählt von
(I) Kontaktieren/Inberührungbringen des Collagens mit einem querverbindenden Mittel, ausgewählt von der Gruppe bestehend aus Carbodiimid und bifunktionalem Suiccinimidyl aktiven Estern,
(II) Aussetzen bzw. Unterwerfen der festen Tröpfchen erhöhten Temperaturen unter Vakuum und
(III) einer Kombination hiervon.

12. Mikroschwamm nach Anspruch 11, wobei das inerte Gewichtsmaterial als fein verteiltes Pulver vor der Gefriertrocknung in die Mischung von Collagen und saurem, flüssigem Mittel zugesetzt wird.

13. Der Mikroschwamm nach Anspruch 12, wobei das Collagen vom Typ 1 ist, Tendon Collagen.

14. Ein Bioreaktor-System bestehend aus einer Vielzahl beschwerter Mikro-Schwämme gemäß Anspruch 1, die in einem Reaktorbehälter sterilisiert und aseptisch versiegelt sind.

15. Ein Bioreaktor-System gemäß Anspruch 14, wobei der Reaktor ein Volumen zwischen 0,1 bis 10 Litern hat.

16. Ein Bioreakter-System gemäß Anspruch 15, wobei der Reaktor ein Fluid-Bett-Reaktor mit einer Fluidverteilungsplatte ist.

17. Ein Verfahren zur Ausführung einer Bioreaktion, umfassend eine Immobilisierung eines bioaktiven Materials in Mikroschwämmen gemäß Anspruch 1; Einbringen der Mikroschwämme mit dem Immobilisierten bioaktiven Material in einen passenden Reaktorbehälter; Durchleiten eines flüssigen Reaktionsmittel-Stromes in und durch den Reaktor in direktem Kontakt mit den Mikroschwämmen, Rühren der Mischung aus Mikroschwämmen und strömendem Reaktionsmittel, Gewinnen des biochemischen Reaktionsproduktes aus dem Reaktor.

18. Ein Verfahren gemäß Anspruch 17, wobei Organismen in den Mikroschwämmen immobilisiert sind, wobei die Mikroschwämme vorbereitet/ausgebrütet sind zur Förderung des Wachstums und zur Colonisierung der Mikroschwämme durch die Organismen, wobei das Reaktionsmittel Nährmittel zur Förderung des Wachstums und der Metabolisierung der Organismen enthält, und wobei das Produkt Metabole (Stoffwechselprodukte) der Organismen enthält.

19. Ein Verfahren nach Anspruch 17, wobei die Organismen an bzw. auf den Mikroschwämmen immobilisiert sind und das gewonnene Produkt freie Organismen enthält, die von den Mikroschwämmen entwichen sind.

20. Ein Verfahren nach Anspruch 18, wobei die Organismen aufweisen bzw. bestehen aus Hybridomas und das Produkt monoclonale Antikörper aufweist.

21. Verfahren nach Anspruch 20, wobei der Reaktorbehälter einen Fluid-Bett-Reaktor aufweist.

22. Verfahren nach Anspruch 18, wobei die Organismen Säugetierzellen und das Produkt Säugetierzellprodukte umfassen.

23. Ein Verfahren nach Anspruch 18, wobei die Organismen durch Gentechnik hergestellte Mikro-Zellen sind und das Produkt ausgeschiedene Proteinprodukte umfaßt.

24. Ein Verfahren nach Anspruch 19, wobei die Organismen durch Gentechnik hergestellte Mikroben-Zellen sind und das Produkt Organismen umfaßt, welche ein nicht ausgeschiedenes Protein-Produkt enthält.

25. Ein Verfahren für die kontinuierliche In-Vitro-Kultur von Organismen für die Herstellung biochemischer Produkte, umfassend die Schritte:
(a) Bereitstellung eines hochgradig querverbundenen Collagen Mikroschwammes, der hergestellt wurde durch
(I) Mahlen einer Quelle von Collagen, welches ausgewählt ist von der Gruppe bestehend aus den Typen I, II und III Collagenen,
(II) Mischen des gemahlenen Collagens mit einem sauren, flüssigen Medium,
(III) Gefriertrocknen der Collagen-Flüssigkeitsmischung zu einer trockenen schwammigen Matrix und
(IV) Querverbindung des Collagens in der Schwamm-Matrix durch eine Behandlung ausgewählt aus
(1) Inkontaktbringen des Collagens mit einem Querverbindungselement, ausgewählt aus der Gruppe bestehend aus Carbodiimiden und bifunktionalen Succinimidyl aktiven Estern,
(2) Unterwerfen der trockenen schwammigen Matrix erhöhten Temperaturen und einem Vakuum, und
(3) einer Kombination davon;
(b) Impfen des hoch querverbundenen Collagen-Mikroschwammes der Stufe (a) mit einer Kultur des Mikro-Organismus;
(c) Durchleiten einer Nährlösung in direktem Kontakt mit dem geimpften, hochgradig querverbundenen Collagen-Mikroschwammes und
(d) Gewinnung des biochemischen Produktes aus der abfließenden Nährlösung.

26. Ein Verfahren nach Anspruch 25, wobei die Organismen ausgewählt sind aus Bakterien, Pilzen, Viren, Algen, Hefen, tierischen Zellen und pflanzlichen Zellen.

27. Ein Verfahren nach Anspruch 25, wobei die Organismen Säugetierezellen sind und die biochemischen Produkte Säugetierzell-Produkte umfassen.

28. Ein Verfahren nach Anspruch 25, wobei die Organismen Hybridomas sind und die biochemischen Produkte monoclonale Antikörper umfassen.

29. Ein Verfahren gemäß Anspruch 25, wobei die Organismen gentechnisch hergestellt sind und die biochemischen Produkte Organismen umfassen, die ein nicht ausgeschiedenes Protein-Produkt enthalten.

30. Ein Verfahren gemäß Anspruch 25, wobei die Organismen gentechnisch behandelt/hergestellt sind und die biochemischen Produkte ausgeschiedene (sekretierte) Protein-Produkte umfassen.

31. Ein Verfahren gemäß Anspruch 25, wobei der hochgradig querverbundenen Collagen-Mikroschwamm gebildet ist in einer Form ausgewählt aus Perlen, Flocken, Scheiben, Basen, Filmen und Bogen.

32. Ein Verfahren gemäß Anspruch 25, wobei der hochgradig querverbundene Collagen-Mikroschwamm vorgesehen ist als ein Überzug auf einem Träger.

33. Ein Verfahren gemäß Anspruch 25, wobei der hochgradig querverbundene Collagen-Mikroschwamm eine zu den Oberflächen offene Porenstruktur hat mit einer durchschnittlichen Porengröße im Bereich von ungefähr 1 bis ungefähr 150 Mikrons und die Poren des Mikroschwammes ungefähr 70 bis ungefähr 98 Vol.-% der Matrix einnehmen.

34. Ein Verfahren gemäß Anspruch 25, wobei der hochgradig querverbundene Collagen-Mikroschwamm vorliegt in der Gestalt von Perlen mit einer durchschnittlichen Partikelgröße von ungefähr 100 bis 1000 Mikron.

35. Ein Verfahren nach Anspruch 25, wobei das Nährmedium durchgeleitet wird in direktem Kontakt mit den ungeimpften, hochgradig querverbundenem Collagen-Mikroschwamm in einem Reaktor ausgewählt aus Fest-Bett-Reaktoren, Rührtank-Reaktoren, Fluid-Bett-Reaktoren und Bewegt-Bett-Reaktoren.

36. Ein Verfahren gemäß Anspruch 25, wobei die hochgradig querverbundene Collagen in dem Mikroschwamm ein Molekulargewicht von ungefähr 1×10^6 bis ungefähr 50×10^6 hat.

37. Ein Verfahren gemäß Anspruch 25, wobei das hochgradig querverbundene Collagen zwischen den Querverbindungen ein Molekulargewicht von ungefähr 1000 bis 100000 Kovalenzbindungen (Elektronenpaarbindungen) aufweist.

Revendications

1. Une microéponge permettant d'immobiliser des matières bio-actives dans les systèmes de

bio-réacteurs ouverts, caractérisée en ce que la microéponge comprend une matrice biostable poreuse constituée d'un polymère biocompatible, comprenant un matériau alourdissant inerte qui alourdit la micro-éponge; la matrice présentant une ouverture au niveau de la surface poreuse avec une dimension moyenne des pores comprise dans un intervalle allant d'environ 1 à environ 150 microns, les pores de la matrice occupant environ 70 à environ 98% en volume de la micro-éponge, et en ce que la micro-éponge a une taille moyenne de particules d'environ 100 à 1000 microns et une densité spécifique d'environ 1,05.

2. Une microéponge selon la revendication 1, dans laquelle sont immobilisées les matières bio-actives sélectionnées dans le groupe consistant en : enzymes, micro-organismes, cellules mortes et cellules vivantes.

3. Une microéponge selon la revendication 1, caractérisée en ce que le polymère bio-compatible bio-stable est sélectionné parmi collagène, cellulose, dextran, dextrine, polyamides, polyesters, amidon, agarose, carragénine, polyuréthanes, alcools polyvinyliques, polyacrylates, polyméthacrylates et polyacrylamides.

4. Une microéponge selon la revendication 1, caractérisée en ce que le matériau alourdissant inerte est sélectionné parmi des métaux, des alliages de métaux, des oxydes métalliques et des céramiques.

5. Une microéponge selon la revendication 4, caracté risée en ce que le matériau alourdissant a une densité spécifique supérieure à environ 4,0 et la microéponge a une densité spécifique supérieure à environ 1,3.

6. Une microéponge selon la revendication 5, caractè risée en ce que le matériau alourdissant inerte est dispersé de façon homogène dans la matrice poreuse sous la forme d'une poudre finement divisée.

7. Une microéponge selon la revendication 5, caractérisée en ce que le matériau alourdissant est disposé au centre sous la forme d'un noyau solide autour duquel la matrice poreuse est formée.

8. Une microéponge selon la revendication 5, caractérisée en ce que le matériau alourdissant inerte est sélectionné parmi chrome, tungstène, cobalt, molybdène, titane, nickel et les alliages.

9. Une microéponge selon la revendication 8, caractérisée en ce que le matériau alourdissant est du titane et des cellules d'hybridomes sont immobilisées dans la microéponge.

10. Une microéponge selon la revendication 1, caractérisée en ce que le polymère bio-compatible bio-actif correspond à du collagène hautement réticulé.

11. Une microéponge selon la revendication 10, caractérisée en ce que la matrice de collagène hautement réticulé est préparée par les étapes suivantes :
    (a) broyage d'une source de collagène sélectionnée parmi les collagènes du type 1, 2 et 3,
    (b) mélange du collagène broyé avec un milieu liquide acide,
    (c) liophilisation du mélange milieu liquide-collagène dans une matrice sèche, et
    (d) réticulation du collagène dans la matrice sèche par un traitement sélectionné parmi
        (i) la mise en contact du collagène avec un agent de réticulation sélectionné dans le groupe consistant en carbodiimides et esters actifs bifonctionnels de suiccinimidyl,
        (ii) l'exposition des particules solides à des températures élevées sous vide et,
        (iii) une combinaison de ces traitements.

12. Une microéponge selon la revendication 11, caractérisée en ce que le matériau alourdissant inerte est ajouté au mélange de collagène et de milieu acide sous la forme d'une poudre finement divisée avant l'étape de liophilisation.

13. La microéponge selon la revendication 12, caractérisée en ce que le collagène est un collagène de tendon de type 1.

14. Un système de bio-réacteur comprenant une pluralité d'éponges chargées, selon la revendication 1, stérilisées et scellées de façon aseptique dans le récipient du réacteur.

15. Un système de bio-réacteur selon la revendication 14, caractérisé en ce que le réacteur a un volume compris entre environ 0,1 et 10 litres.

16. Un système de bio-réacteur selon la revendication 15, caractérisé en ce que le réacteur est un réacteur à lit fluidisé comportant une plaque de distribution du fluide.

17. Un procédé permettant de réaliser une bio-réaction comprenant l'immobilisation d'une matière bio-réactive dans des microéponges selon la revendication 1; le chargement des microéponges comportant la matière du réactif immobilisé dans un récipient de réacteur approprié; le passage d'un courant de réactif dans le réacteur au contact direct avec les microéponges; l'agitation du mélange des microéponges et du courant de réactif et la récupération des produits de réaction biochimique à partir du réacteur.

18. Un procédé selon la revendication 17, caractérisé en ce que des microorganismes sont immobilisés dans les microéponges, les microéponges sont incubées pour favoriser la croissance et la colonisation des microéponges par les microorganismes, le réactif comprend un milieu nutritif permettant de favoriser la croissance et le métabolisme des microorganismes, et en ce que le produit comprend les métabolites des microorganismes.

19. Un procédé selon la revendication 17, caractérisé en ce que des microorganismes sont immobilisés sur les microéponges, et le produit récupéré comprend les microorganismes libres qui se sont échappés des microéponges.

20. Un procédé selon la revendication 18, caractérisé en ce que les microorganismes comprennent des hybrodomes et le produit comprend des anticorps monoclonaux.

21. Un procédé selon la revendication 20 caractérisé en ce que le récipient du réacteur comprend un réacteur à lit fluidisé.

22. Un procédé selon la revendication 18, caractérisé en ce que les microorganismes comprennent des cellules de mammifères et les produits comprennent des produits de cellules de mammifères.

23. Un procédé selon la revendication 18, caractérisé en ce que les microorganismes sont des cellules microbiennes génétiquement modifiées et le produit comprend des produits protéiques sécrétés.

24. Un procédé selon la revendication 19, caractérisé en ce que les microorganismes sont des cellules microbiennes génétiquement modifiées et le produit comprend des organismes contenant un produit protéique non-sécrété.

25. Un procédé permettant la culture in vitro en continu d'organismes pour la production de produits biochimiques comprenant les étapes suivantes :
    (a) utilisation d'une microéponge à base de collagène hautement réticulé préparé par :
        (i) broyage d'une source de collagène sélectionnée dans le groupe consistant en collagène de type 1, 2 et 3,
        (ii) mélange du collagène broyé avec un milieu liquide acide,
        (iii) liophilisation du mélange milieu liquide-collagène dans une matrice d'éponge sèche, et
        (iv) réticulation du collagène dans la matrice d'éponge par un traitement sélectionné parmi :
            (1) la mise en contact du collagène avec un agent de réticulation sélectionné dans le groupe consistant en carbodiimides et les esters actifs bifonctionnels de succinimidyl,
            (2) l'exposition de la matrice d'éponge sèche à des températures élevées sous vide, et
            (3) une combinaison de ces traitements;
    (b) inoculation de la microéponge à base de collagène hautement réticulée de l'étape (a) avec une culture de microorganismes;
    (c) passage d'un milieu nutritif au contact direct de la microéponge inoculée à base de collagène hautement réticulé; et
    (d) récupération des produits biochimiques avec l'effluent de milieu nutritif.

26. Un procédé selon la revendication 25, caractérisé en ce que les microorganismes sont sélectionnés parmi bactéries, champignons, virus, algues, levures, cellules animales et cellules végétales.

27. Un procédé selon la revendication 25, caractérisé en ce que les microorganismes sont des cellules de mammifères et les produits chimiques comprennent des produits de cellules de mammifères.

28. Un procédé selon la revendication 25, caractérisé en ce que les microorganismes sont des hybridomes et les produits biochimiques comprennent des anticorps monoclonaux.

29. Un procédé selon la revendication 25, caractérisé en ce que les microorganismes sont modifiés génétiquement et les produits biochimiques comprennent les organismes contenant un produit protéique non-sécrété.

30. Un procédé selon la revendication 25, caractérisé en ce que les organismes sont modifiés génétiquement et les produits biochimiques comprennent des produits protéiques sécrétés.

31. Un procédé selon la revendication 25, caractérisé en ce que la microéponge à base de collagène hautement réticulé est formée sous une forme sélectionnée parmi billes, flocons, disques, fibres, films et feuilles.

32. Un procédé selon la revendication 25, caractérisé en ce que ladite microéponge à base de collagène hautement réticulé est préparée sous la forme d'un revêtement sur un support.

33. Un procédé selon la revendication 25, caractérisé en ce que la microéponge à base de collagène hautement réticulé présente une ouverture au niveau de la surface poreuse avec une dimension moyenne des pores comprise dans un intervalle allant d'environ 1 à environ 150 microns, et les pores de la microéponge occupent environ de 70 à environ 98% en volume de la matrice.

34. Un procédé selon la revendication 25, caractérisé en ce que la microéponge à base de collagène hautement véhiculé est sous forme de billes ayant une dimension particulaire moyenne d'environ 100 à 1000 microns.

35. Un procédé selon la revendication 25, caractérisé en ce que le milieu nutritif passe au contact direct de la microéponge inoculée à base de collagène hautement réticulé dans un réacteur sélectionné parmi des réacteurs à lit fixe, des réacteurs agités, des réacteurs à lit fluidisé et des réacteurs à lit mobile.

36. Un procédé selon la revendication 25, caractérisé en ce que le collagène hautement réticulé présent dans la microéponge a un poids moléculaire compris entre environ 1.10^6 et environ 50.10^6.

37. Un procédé selon la revendication 25, caractérisé en ce que le collagène hautement réticulé a un poids moléculaire entre les liaisons de réticulation d'environ 1000 à environ 100000 par liaison covalente.

FIG. 1.

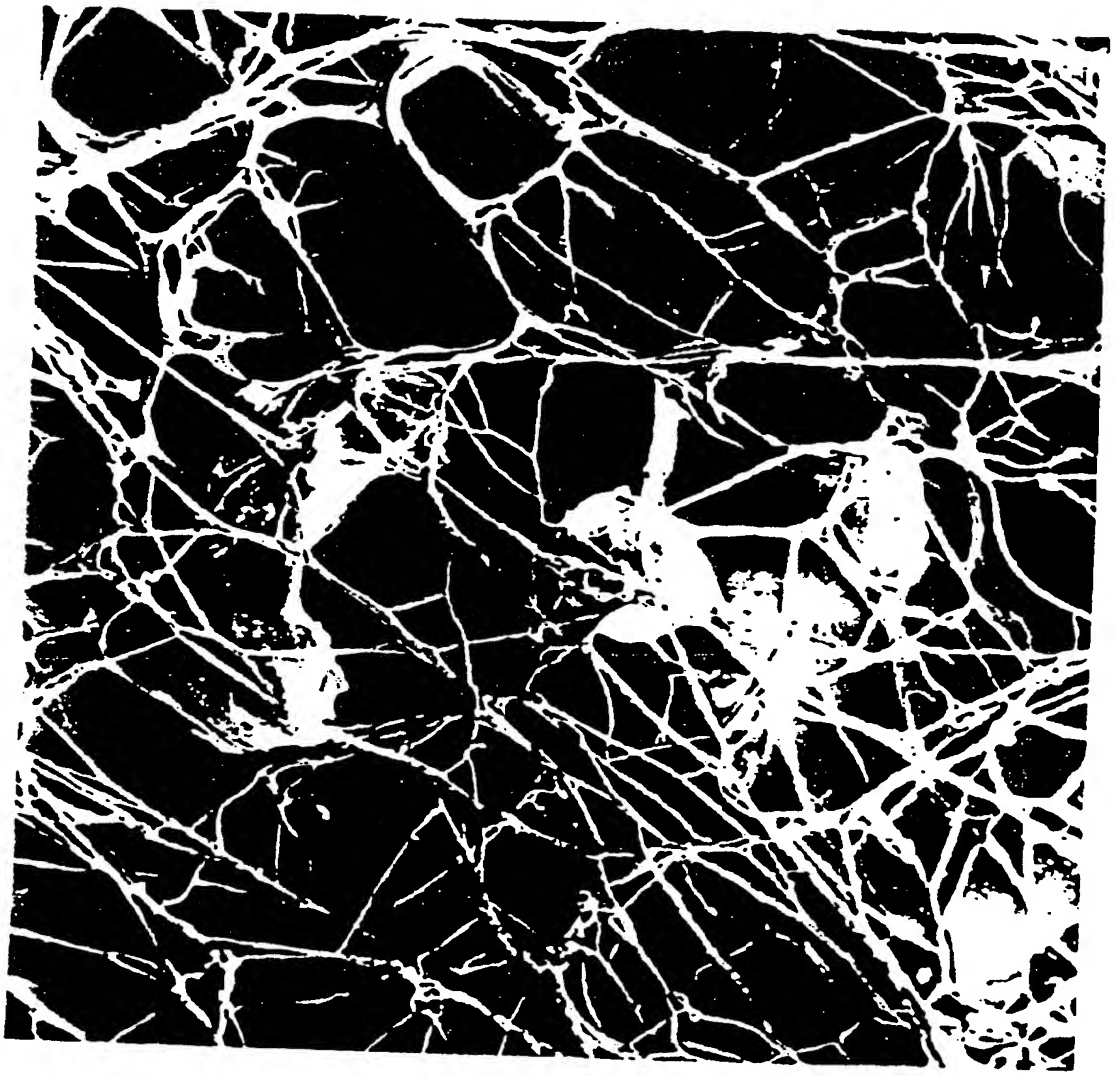

FIG. 2.